

**The clinical spectrum of acute hepatitis E infection in patients  
with chronic liver disease in a tertiary level institution**

**A dissertation submitted in part fulfillment of DM  
(Gastroenterology) examination of the Tamil Nadu  
Dr. MGR Medical University, Chennai to be held in  
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# CERTIFICATE

This is to certify that this dissertation entitled ` **The clinical spectrum of acute hepatitis E infection in patients with chronic liver disease in a tertiary level institution** ' is a bonafide work done by Dr. Uday George Zachariah in partial fulfillment of the rules and regulations for DM (Gastroenterology) examination of the Tamil Nadu Dr. MGR Medical University, to be held in February 2007.

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# INDEX

	PAGE NO.
<b>INTRODUCTION</b>	<b>1</b>
<b>AIMS</b>	<b>3</b>
<b>REVIEW OF LITERATURE</b>	<b>4</b>
<b>METHODOLOGY</b>	<b>33</b>
<b>RESULTS</b>	<b>37</b>
<b>DISCUSSION</b>	<b>51</b>
<b>CONCLUSIONS</b>	<b>57</b>
<b>BIBLIOGRAPHY</b>	
<b>APPENDIX</b>	

## **INTRODUCTION**

Hepatitis E virus (HEV) infection is a major public health problem in several developing countries of the world, especially the Indian sub-continent, South East and Central Asia, where it is endemic.<sup>1,2,3</sup> HEV in endemic regions not only causes large epidemics but is also responsible for a significant proportion of acute sporadic hepatitis in both children and adults. In India, HEV infection alone has been shown to account for 50-70% of all episodes of sporadic viral hepatitis.<sup>4,5</sup> It has been suggested that HEV is more widespread than previously believed, prompting the World Health Organization to label HEV as an “emerging pathogen”.

Acute HEV infection is generally a self limiting illness similar to other forms of acute viral hepatitis, except in pregnant women in whom the illness is particularly severe with a high mortality rate.<sup>6,7,8</sup> Accumulating evidence indicates that HEV infection may be associated with severe liver disease in certain other settings also, namely, concomitant HEV and HAV infection in children<sup>9</sup>, acute liver failure in the sporadic setup<sup>10</sup> and superinfection on preexisting chronic liver disease (CLD).<sup>11,12,13,14,15</sup> Therefore the disease burden of HEV in the developing world is heavy as it causes substantial morbidity, mortality and economic loss.<sup>16</sup>

Acute on chronic liver failure (ACLF) is a poorly defined entity which has been described by Jalan et al as an acute deterioration in liver function over a period of two to four weeks in patients with previously well compensated CLD, caused by precipitating events such as sepsis, upper gastrointestinal bleed, ischaemia or superimposed liver

injury due to alcohol, hepatotoxic drugs or hepatitis virus infection.<sup>17</sup> The role of viral hepatitis infections in the genesis of ACLF gains significance in countries like India which are hyperendemic for the enterically transmitted hepatotropic viruses, hepatitis A virus (HAV) and HEV.

Superinfection with HAV in CLD, particularly chronic hepatitis C associated liver disease has been shown to result in exacerbation of the underlying CLD with high mortality.<sup>18</sup> Hence, HAV vaccination is advocated in patients with CLD in non endemic areas. Since HEV shares several epidemiological similarities with HAV including similar dynamics of disease transmission, superimposed HEV infection in patients with previously stable CLD merits critical attention as another preventable cause of severe liver decompensation. But the data on HEV superinfection in patients with underlying CLD is limited to a few small case series from the Indian sub-continent.<sup>11,12,13,14</sup> There are unresolved issues regarding the behaviour of HEV in CLD. More data is needed to confirm its role in causation, persistence and final outcome of severe liver failure. A set of indicators of poor prognosis needs to be determined in this group of patients in order to define the best therapeutic strategies. Hence the present study was conceived in an attempt to better understand the clinical presentation, outcome and prognostic factors of acute HEV superinfection in CLD.

**AIM**

1. To study the clinical profile and outcome of acute hepatitis E virus superinfection in patients with chronic liver disease.
2. To identify factors affecting the outcome and determine their prognostic significance.

## **REVIEW OF LITERATURE**

### **Hepatitis E virus**

#### ***Historical background:***

Hepatitis E, previously known as enterically transmitted non-A, non-B hepatitis is an infectious viral disease with clinical and morphological features of acute hepatitis. The disease was first recognized as a distinct clinical entity in the 1980s, when sera from persons affected during a large waterborne epidemic of acute hepatitis during 1955-56 in Delhi, India and another epidemic in Kashmir, India tested negative for serological markers of acute hepatitis A and B.<sup>6,7,19</sup> The occurrence of the first recorded epidemic of hepatitis E as late as 1955 and the infrequency of this disease in developed countries suggest that hepatitis E is a new emerging infectious disease.

The first proof of the existence of a novel viral hepatitis agent was obtained in 1983, when virus like particles were detected by immune electron microscopy in faeces collected from a volunteer who was infected with faecal material from patients with suspected enterically transmitted non A, non B hepatitis.<sup>20</sup> The disease was successfully transmitted to cynomolgus monkeys who excreted similar virus like particles in their faeces.<sup>20</sup> The genome of this virus, now known as HEV<sup>21</sup> was cloned in 1990<sup>22</sup> and fully sequenced shortly thereafter.<sup>23,24</sup>

### ***Epidemiology:***

Regions of the world can be considered as hepatitis E disease endemic or non endemic regions based on the periodic occurrence of disease outbreaks. The geographical distribution of HEV is shown in figure 1.

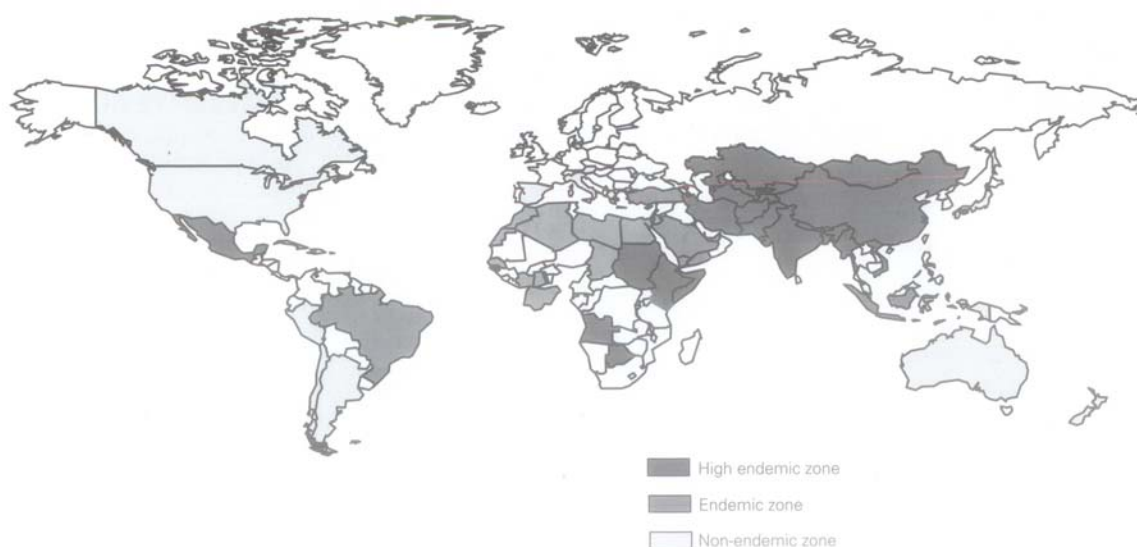


Figure 1: Geographical distribution of HEV

The Indian subcontinent has faced many explosive outbreaks of the disease during the last four decades. The first and the best studied epidemic of HEV was reported in 1957 in which 29,000 people in Delhi were affected between December 1955 and January 1956.<sup>7</sup> Subsequently eight well studied epidemics (table I) have been reported from India with uniformly similar epidemiological features documented.<sup>25-30</sup>



<i>Year</i>	<i>Location</i>	<i>number</i>	<i>Fatality%</i>	<i>Aetiology</i>
1955 (Vishwanathan)	Delhi	29300	0.22 (10.5%)#	HEV**
1960 (Bhattacharjee)	Kharagpur	65	0.0 (NA)	HEV*
1961 (Dhamdhere)	Aurangabad	865	0.34 (NA)	HEV*
1966 (Pattanayak)	Siliguri	4287	0.09 (NA)	HEV*
1975 (Sreenivasan)	Ahmedabad	2572	2.4 (NA)	HEV**
1978 (Khuroo)	Kashmir	275	3.6 (75%)#	HEV***
1979 (Tandon)	Azamgarh	152	12.0 (39%)#	HEV**
1990 (Naik)	Kanpur	79091	0.06 (NA)	HEV**

\* presumed aetiology (serological studies not carried out)

\* \* absence of serology for HBV / HAV

\* \* \* positive serology for HEV

# mortality in pregnancy

Several large epidemics of HEV have been observed in the developing countries of South East and Central Asia.<sup>3,6,7,8</sup> Outbreaks have been documented in the Middle East and Northern and Western parts of Africa. Two small outbreaks were reported from Mexico, North America in the year 1986-87.<sup>31, 32</sup>

#### *HEV in disease endemic areas:*

In disease endemic regions the main epidemiological features which characterize HEV infection are as follows:

- The HEV outbreaks are large,<sup>3,6,7,8</sup> frequently affecting several hundreds to thousand persons in developing countries. Their time course varies from short lived single peaked outbreaks lasting a few weeks to prolonged, multimodal

epidemics lasting over a year. The outbreaks recur with a periodicity of 5-10 years.

- HEV infection accounts for a substantial proportion of acute sporadic hepatitis in both children and adults. In India, HEV infection accounts for 50-70% of all patients with sporadic viral hepatitis.<sup>4,5</sup> Demographic and clinical features of patients with sporadic hepatitis E closely resemble those of epidemic hepatitis E.<sup>33</sup>
- The fecal-oral route is the predominant route of transmission of epidemic HEV infection. Consumption of faecally contaminated drinking water has been incriminated as the cause of most reported outbreaks.<sup>6,7,8</sup> Such contamination was due to backflow of sewage during floods,<sup>8,30</sup> leakage of sewage drains contaminating corroded drinking water pipes<sup>7</sup> and contamination of well water during rain.<sup>25,26,27</sup> Food borne transmission has been postulated in some small outbreaks in China which occurred after community feasts.<sup>3</sup>
- Person to person transmission of HEV infection is distinctly uncommon unlike other faeco-orally transmitted viral infections.<sup>7,34</sup> Secondary attack rates among house hold contacts of HEV infected individuals are only 0.7-2.2%<sup>7,35</sup> in contrast to 50-75% in the case of HAV.<sup>36</sup>
- Vertical transmission of HEV infection from mother to infant has been reported. Presence of HEV RNA was documented in five of six babies born to mothers with HEV infection in the third trimester.<sup>37</sup>

- The probability of nosocomial spread of HEV has been raised from South Africa where three health care workers who treated a patient with fulminant HEV infection, developed HEV infection six weeks later.<sup>38</sup>
- There is no evidence of sexual transmission of HEV.
- Parenteral transmission of HEV has been documented in a recent study demonstrating the presence of HEV viraemia among healthy blood donors and transmission of infection to transfusion recipients.<sup>39</sup>
- During epidemics, overall attack rates range from 1-15%. The highest attack rates is seen among young adults aged 15-40 years (3-30%) with relative sparing of children below 14 years.<sup>7,8,25,26,27,30</sup> However in the sporadic setting, overt icteric hepatitis has been well documented in children.<sup>40,41</sup> During epidemics, anicteric hepatitis was found to be more frequent than icteric hepatitis.<sup>7,8,25,26,27,30</sup>
- The male to female ratio among cases in most reports varies from 1:1 to 4:1.
- HEV outbreaks are characterized by a particularly high attack rate and mortality (15-25%) among pregnant women, especially those in the third trimester.<sup>42,43</sup> The frequency of acute liver failure has been significantly higher among pregnant women with HEV infection (10-22%) than the non pregnant women and men with icteric hepatitis(1-2%).<sup>7,8,25-27,30</sup>

*HEV in disease non endemic areas:*

HEV accounts for less than 1% of acute viral hepatitis in non endemic regions and indigenous transmission of the virus in these areas appears to be rare. Isolated sporadic cases occur mainly among travellers to endemic regions.<sup>44,45</sup>

*Reservoirs of HEV:*

Subclinical HEV infections that maintain the presence of the virus in a population during inter epidemic periods may be a potential reservoir of HEV in endemic regions. In the experimental model of HEV infection in cynomolgus macaques, animals with HEV infection without biochemical evidence of liver injury excreted large amounts of the virus.<sup>46</sup> However more data is needed to confirm the existence of continuous sub clinical circulation of HEV in endemic areas.

*Is Hepatitis E a zoonotic disease? :*

There is increasing evidence to implicate a zoonotic reservoir in the epidemiology of HEV which is as follows:

- Discovery of swine HEV in pigs closely related to human HEV.<sup>47</sup>
- Identification of genotype 3 & 4 human HEV isolates genetically identical to swine HEV isolates in the same geographic regions.<sup>48-57</sup>
- Experimental cross-species infection of non human primates by swine HEV and of pigs by human HEV.<sup>58</sup>
- Significantly higher anti HEV antibody prevalence in pig handlers than in age and geography matched controls.<sup>50,51,59,60</sup>

- Epidemiological link of sporadic cases of acute HEV to consumption of undercooked pork livers and detection of near identical HEV sequences from patients and packaged pork livers sold in local grocery stores.<sup>56,57</sup>
- Existence of numerous other animal species positive for anti HEV antibodies (chicken, monkeys, cats , dogs, cattle and rodents).<sup>61,62,63</sup>
- Discovery of avian HEV from bile samples of chickens with hepatitis splenomegaly syndrome in USA which is antigenically and genetically related to human HEV. Sequence data suggests that the virus causing big liver and spleen disease (BLSV) is also genetically related.<sup>63-66</sup>
- Experimental cross-species infections of lambs and rodents by human HEV.<sup>62</sup>
- Sporadic cases of acute HEV linked to consumption of raw deer meat.<sup>67</sup>
- Significantly higher anti HEV antibody prevalence in humans with occupational exposure to animals than in controls.

However the existing available data is insufficient to support the hypothesis of universal zoonotic origin of HEV. Moreover, genotypes 1 and 2 (responsible for large human epidemics) failed to induce infection in experimentally inoculated pigs.<sup>68</sup>

### ***Classification:***

HEV was originally classified within the Caliciviridae family but more detailed sequence analysis has resulted in its removal from this family and HEV is now classified as the type species of the genus Hepevirus, family Hepeviridae in the 8<sup>th</sup> ICTV report.<sup>69</sup>

Recent studies have greatly expanded the known diversity of HEV to include 4 putative genotypes.<sup>70</sup> The prototype Burmese and related strains (Chinese) are classified as genotype 1, the divergent Mexican strain is classified as genotype 2, the swine HEV strain and closely related strains are classified as genotype 3 and distinct isolates from patients in China and Taiwan are classified as genotype 4.

***Structure and assembly:***

Virions of HEV isolated from bile or faeces are non enveloped, icosahedral particles of approximately 32-34 nm diameter,<sup>71</sup> comprising a single viral protein, which encapsidates the single stranded positive sense RNA genome of approximately 7200 nucleotides. Naked particles demonstrate some limited surface morphology but this is generally obscured by the use of antibody to complex virions for electron microscopy. (figure 2)

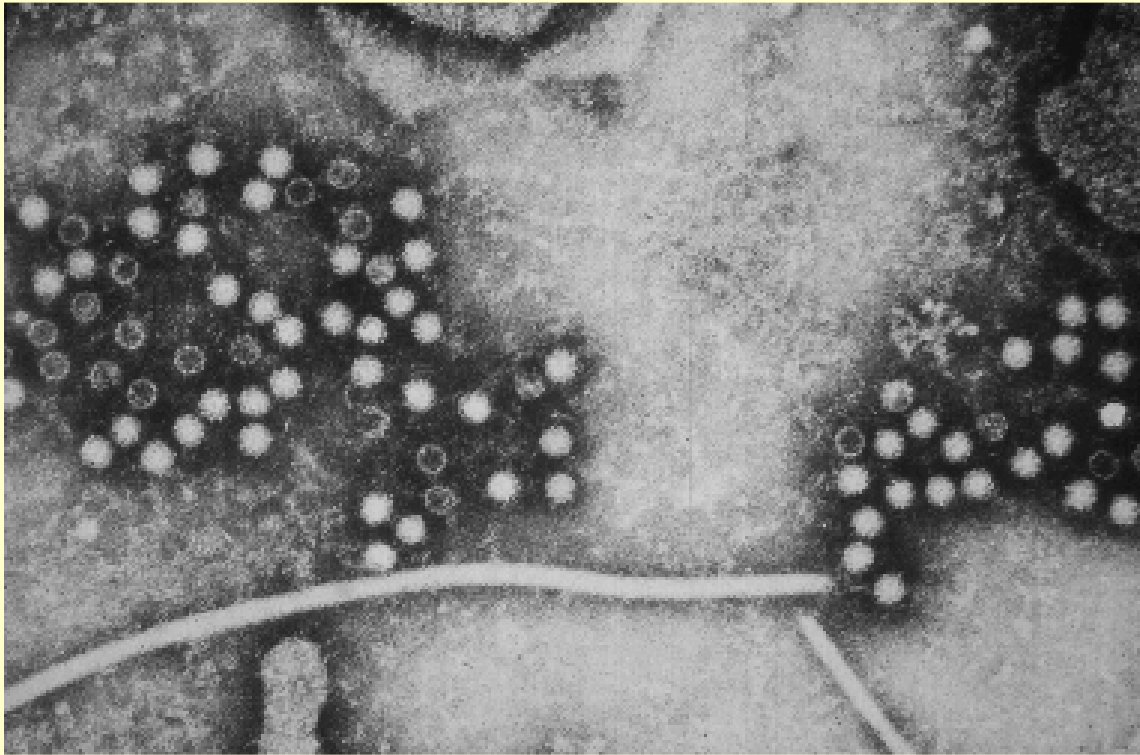


Figure 2: HEV particles from infected macaque faeces, complexed with acute phase patient serum.

The 7204 nucleotide infectious cDNA clone of the SAR-55 strain of HEV described by Emerson and colleagues can be assumed to be full length.<sup>72</sup> Most isolates of HEV are close to this length. The HEV genome contains a 7-methyl guanosine cap at the 5' end,<sup>73</sup> essential for infectivity of RNA transcribed from cDNA.<sup>72</sup> The viral RNA has short, highly conserved 5' and 3' untranslated regions (UTRs) of 25 and 68-75 nucleotides respectively which are likely to function in RNA replication and / or encapsidation. Three open reading frames (ORFs) organized as 5' – ORF1 – ORF3 – ORF2 - 3' with ORF3 and ORF2 largely overlapping, encode the viral proteins. The viral proteins (PORFs) may be translated from a set of sub genomic mRNA molecules. The PORF1 polyprotein contains domains consistent with replicative proteins. The PORF2 is the major capsid protein. The

function of PORF3 is unknown but it may have regulatory or replicative functions. Linear antigenic domains have been identified by peptide scanning through out each of the 3 proteins. (figure 3)

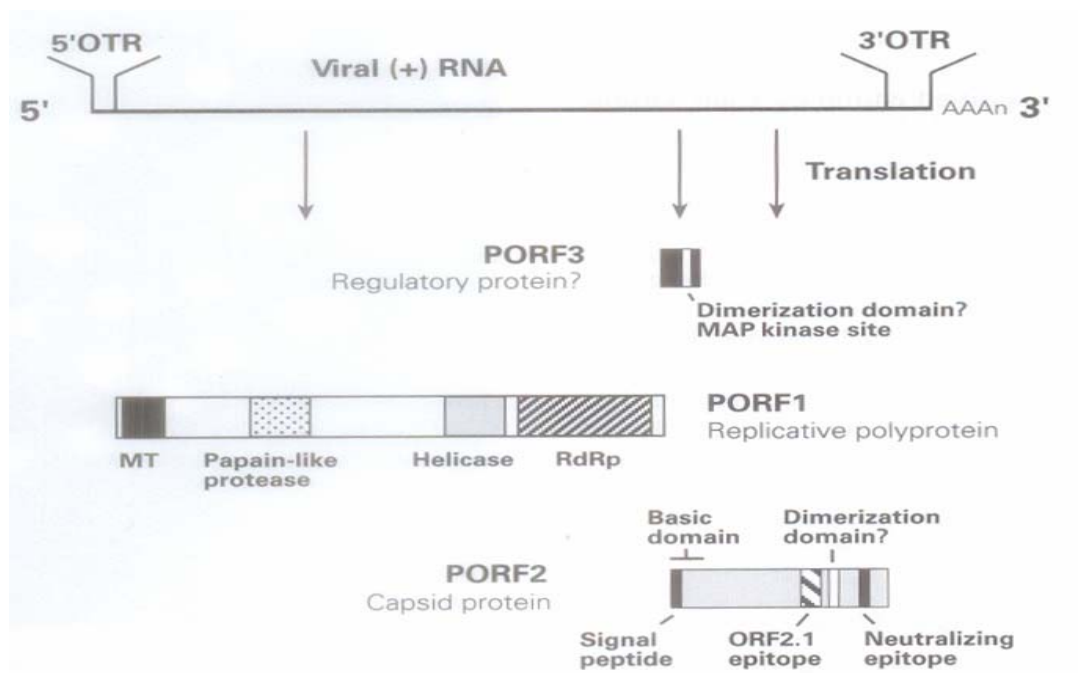


Figure 3: Genome organization and protein domains of HEV

HEV virus like particles (VLPs) are smaller than the intact virus particle but Cheng and colleagues have used cryoelectron microscopy to give the first indications of HEV structure.<sup>75</sup> A most striking feature of the VLP structure is the presence of dimeric subunits. Results of the cryoelectron microscopy analysis suggest that HEV VLPs are assembled as a  $T = 1$  icosahedral particle containing 30 dimeric subunits of 50 kDa PORF2 with the potential to form an intact virion of the correct size with a  $T = 3$  arrangement of 90 dimeric subunits.<sup>75</sup>



*Physico-chemical properties:*

Virions of HEV are much less stable than those of HAV, being sensitive to conditions such as CsCl and freeze thawing. This lower stability is likely to be a major factor in the low secondary attack rate for HEV. The single capsid protein is encoded by ORF2 expected to yield a protein of 660 aminoacids (aa). However the capsid protein of authentic viral particles has not been characterized.

*Viral proteins:*

ORF1: Translation of ORF1 yields a polyprotein (PORF1) of approximately 186 kDa containing sequence motifs consistent with methyl transferase, papain like protease, RNA helicase and RNA dependent RNA polymerase (RdRp).<sup>23</sup> Expression of PORF1 alone in HepG2 cells or in an invitro translation system failed to demonstrate any proteolytic processing into mature products. But in HepG2 cells transfected with a putative infectious cDNA clone of HEV, proteins could be detected<sup>76</sup> leading to the conclusion that PORF1 processing can only occur in the context of the replication cycle.

ORF2: The capsid protein PORF2 translated as a 660 aa protein includes a predicted N-terminal signal peptide.<sup>76</sup> Deletion of the first 34 aa of PORF2 prevents translocation, glycosylation and surface expression, consistent with the predicted role of aa 1-22 as a signal sequence. PORF2 contains significant neutralizing epitopes in the region 578-607 and a conformational immunodominant epitope spanning 394-468.<sup>77</sup> Individual monoclonal antibodies to this immunodominant ORF2 epitope can block around 60% of total convalescent antibody binding to HEV VLPs.<sup>77</sup> When PORF2 is expressed in insect

cells, it is cleaved into truncated forms, some of which have the ability to cell assemble into VLPs or subviral particles (SVPs)<sup>78</sup>

ORF3: PORF3 may be translated from the same subgenomic RNA as PORF2 or from a “bicistronic” sub genomic RNA as ORF3 overlaps both ORF1 and ORF2.<sup>79</sup> PORF3 is commonly reactive with patient antibody and may have regulatory functions in virus replication or assembly.

### ***Viral replication cycle:***

Due to the lack of practicable cell culture systems for HEV, knowledge of HEV replication is poor. The general outline of HEV replication is only inferred. HEV ingested in water or food reaches the liver via blood either via direct uptake of the inoculum through the gastrointestinal mucosa into the circulation or following one or more rounds of amplification in enterocytes. HEV particles interact with specific receptors on the basolateral domains of hepatocytes, leading to virus penetration and uncoating of the genome within the cell. The input viral RNA then serves as mRNA for the translation of PORF1 which is cleaved to yield the mature replicative proteins. The RdRp / helicase complex copies the input genome to yield full length negative strand RNA which in turn serves as a template for the transcription of positive strand RNA molecules and sub genomic mRNAs.<sup>80</sup> These sub genomic mRNAs are translated to yield further molecules of PORF1, PORF2 and PORF3. PORF2 and new viral genomes assemble together into virions. Mature HEV is finally released from the cell mainly through the apical domains

of hepatocytes. The majority of the virus is excreted through the biliary system into the faeces to complete the transmission cycle. (figure 4)

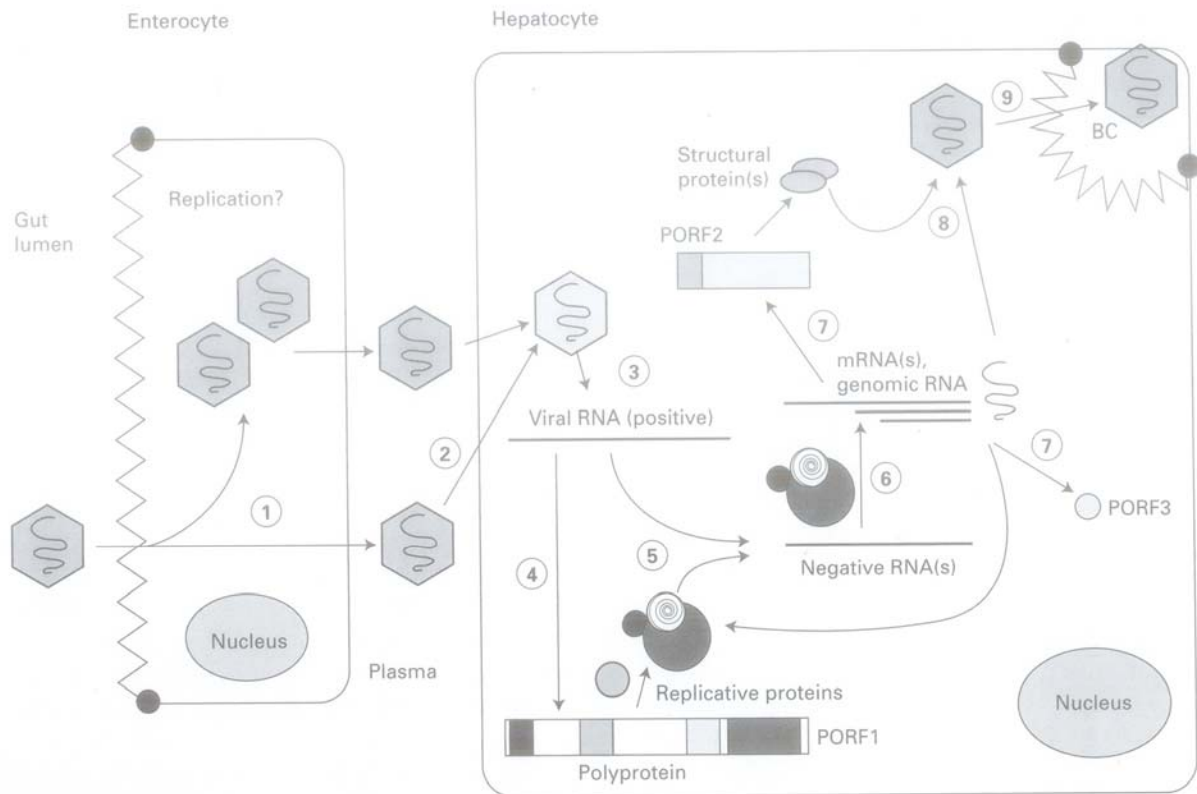


Figure 4: Putative replication cycle of HEV

### ***Pathogenesis:***

The pathogenesis of HEV infection is outlined based on data from human volunteers, patients and experimentally infected animals. The virus enters the host primarily through the oral route. In experimentally infected primates, HEV RNA appears in serum, bile and faeces a few days before the onset of ALT rise. HEV antigen in hepatocytes has been detected simultaneously with HEV identified in bile and faeces during the second or third

week after inoculation. This can be before or concurrent with the onset of ALT elevation and morphological changes in the liver.<sup>81</sup> These findings suggest that HEV may be released from hepatocytes into bile during the initial highly replicative phase of infection before the occurrence of the more prominent histopathological changes in the liver. The onset of ALT elevation and occurrence of pathological changes in the liver generally correspond to the detection of anti-HEV in serum with decreasing levels of HEV antigen in hepatocytes. Both IgM and IgG antibodies have been detected in serum in assays using immunoreactive epitopes of ORF2 and ORF3. The IgM antibody level decreases rather precipitously reaching negligible levels in the early convalescent phase followed by high titres of IgG anti-HEV detected during convalescence. (figure 5)

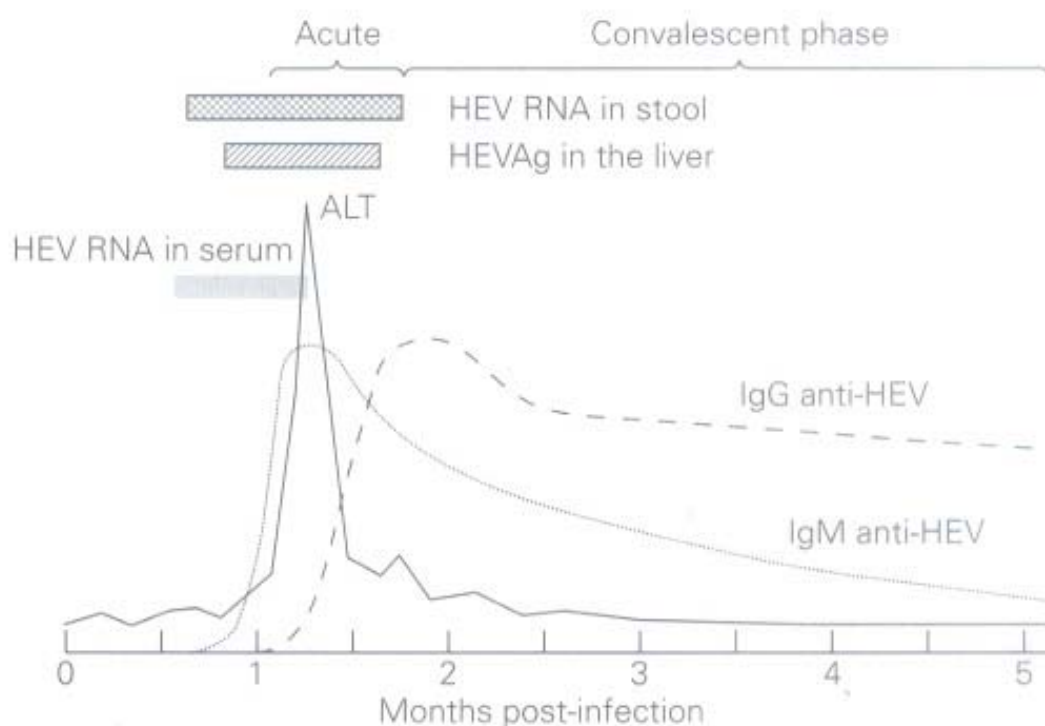


Figure 5: Pathogenetic events in HEV infection

IgM anti HEV appears in the early phase of illness, precedes IgG by a few days and disappears over 4-5 months. IgM anti HEV is positive in more than 90% of patients within 1wk to 2 months after the onset of illness, in 50% at 4months, in 6% at 6-7 months and absent by 8 months. IgG anti HEV which is positive in more than 93% acute hepatitis, persists upto 24 months.

Lymphocytes infiltrating liver in experimentally infected monkeys have a cytotoxic / suppression immunophenotype. Preliminary results of cellular immunity studies in HEV patients indicate that lymphoproliferative responses to HEV peptides from ORF2 and ORF3 regions occur in patients with acute HEV infection.<sup>82</sup> Concordance of pathological, virological and serological findings in HEV suggests that the pathomechanism of the disease may be immune mediated rather than related to cytopathic effect of HEV.

***Clinical and pathological features of HEV:***

The incubation period of HEV ranges from 2-10 weeks. Clinical manifestations are similar to the acute hepatitis of other hepatitis viruses and encompass a wide spectrum of symptoms. Acute icteric hepatitis is the most common recognizable form of illness associated with HEV infection. The illness is usually insidious in onset and has an initial prodromal phase lasting about 1-4 days, with a variable combination of flu like symptoms, anorexia, nausea and vomiting, aversion to smoking, diarrhea, arthralgias, asthenia and a transient macular skin rash. These symptoms are followed in a few days by the appearance of jaundice. The onset of the icteric phase is frequently heralded by the darkening of the urine and may be accompanied by clay stools and itching. Physical

examination reveals a mildly enlarged, soft and slightly tender liver with a soft splenomegaly in a quarter of the patients.

Laboratory test abnormalities include conjugated hyperbilirubinaemia and marked elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) upto 10 times normal and mild rise in serum alkaline phosphatase. The liver enzyme elevation may precede the onset of symptoms by as much as 10 days and reaches a peak by the end of the first week. The liver function test values progressively come back to normal by 6 weeks, initially by decrease in the ALT and AST followed by the bilirubin. The magnitude of transaminase elevation does not correlate well with the severity of liver injury. Some patients show mild leucopenia and relative lymphocytosis. The illness is usually self limiting and typically lasts 1-4 weeks.

Histopathological features of acute HEV infection is similar to that of other forms of acute hepatitis, such as the presence of ballooned hepatocytes and acidophilic bodies, and focal and confluent hepatocyte necrosis with collapse and condensation of the underlying reticulum. Nearly half of the patients have cholestatic hepatitis, which is characterized by canalicular bile stasis and gland-like transformation of the parenchymal cells. In these patients, degenerative changes in hepatocytes are less marked and polymorphonuclear infiltration is prominent. In both forms, lobules and enlarged portal tracts show inflammatory infiltration.

No evidence of chronic hepatitis or cirrhosis has been detected among patients followed up clinically and with liver biopsies after an acute HEV hepatitis.<sup>83,84</sup> A few patients have a prolonged cholestasis which resolves spontaneously in 2-6 months. The prognosis is good in these patients.

HEV infected individuals may develop a non specific acute febrile illness without jaundice (anicteric hepatitis). In its most benign form, an asymptomatic HEV infection passes unnoticed. The exact frequencies of asymptomatic infection and of anicteric hepatitis are not known, but they probably far exceed that of icteric disease.

In a small proportion of patients, disease is more severe and is associated with a sub-acute hepatic failure (SAHF) or fulminant hepatic failure (FHF) that can be rapidly fatal. In patients with severe liver injury, a large proportion of hepatocytes are affected, leading to submassive or massive necrosis and collapse of the liver parenchyma. In disease-endemic regions, hepatitis E is an important cause of FHF. HEV infection (alone or in combination with other hepatitis viruses) in India was responsible for 62% of adult patients and 40% of children with sporadic FHF.<sup>9,85,86,\*</sup> It has also been suggested that FHF and SAHF may result from a combined infection with HBV and HEV.<sup>33,87</sup>

There is also accumulating data on the impact of HEV superinfection in patients with pre-existing CLD. Hamid et al<sup>14</sup> in 2002, documented four cases of HEV superinfection and showed that it may lead to worsening of their clinical state. Subsequently, Jeyamani et al<sup>11</sup> in 2004, retrospectively analysed nine cases of HEV superinfection and found that six

patients had died. The study also noted that six of the nine patients had Wilson's disease as the aetiology of the underlying CLD and that seven patients had presented for the first time with decompensation. Monga et al<sup>13</sup>, over 18 months identified 10 patients with CLD and recent worsening (3 months) of hepatic function who were detected to have acute HEV infection and found that three patients had died. Seven of the ten patients had presented for the first time with decompensation. At the same time, Kumar et al<sup>12</sup> did a prospective case control study with fourteen patients and documented two deaths. All had jaundice for less than one month at presentation as per inclusion criteria. Patients with significant ethanol intake and spontaneous bacterial peritonitis were excluded. The controls in this study were compensated cirrhotics.

### ***Diagnosis:***

One of the earliest tests used for identification of antibodies that react with HEV antigen was the fluorescent antibody blocking assay (FA). Although the FA assay enabled the serologic identification of HEV infection, it does not distinguish between recent and past infections and requires liver tissue substrate that contains HEVAg from HEV infected primates.<sup>81</sup>

Enzyme immunoassays (EIAs) for the detection of IgM and IgG antibodies to HEV have been developed using recombinant HEV antigens expressed in E. Coli or baculovirus in insect cells and synthetic peptides corresponding to immunogenic epitopes of HEV.<sup>88,89</sup> Proteins encoded by all three ORFs of HEV are immunogenic but the ORF2 protein is the most immunogenic, the ORF3 protein is intermediate in immunogenicity and the ORF1



proteins are the least immunogenic. Three major epitopes found in the amino-terminal, central and carboxy-terminal regions respectively of the capsid protein have been reported. A major epitope is found in the carboxy-terminal region of the ORF3 gene product.<sup>90</sup> The most highly conserved epitopes are those found in the capsid protein and thus this protein has been of most interest for the development of diagnostic assays. Antigens that contain the conformational neutralization epitope in the carboxy portion of the ORF2 protein are the most useful for serological studies.<sup>91</sup> Antibodies to the ORF3 protein diminish in titre more rapidly than antibodies to the ORF2 protein. Consequently antibodies to the ORF3 protein are useful for diagnosis of acute HEV infection.

Recombinant peptides expressed from ORF2 and ORF3 of the Mexican strain of HEV were shown to react with sera collected from outbreaks of hepatitis E in Pakistan, Burma, Borneo, the USSR, and Somalia. The existence of these so-called type-common epitopes provided the basis for the development of immunoassays that broadly react with antibodies against different HEV strains. Antigenic domains that contain strong IgG and IgM antigenic epitopes have been identified at the amino and carboxyl terminus of the ORF2 encoded proteins and have been shown to be more sensitive than ORF3 derived antigens, when used for the detection of IgM and / or IgG anti HEV.<sup>92</sup> A highly conserved conformational epitope mapped to 267 aminoacids at the carboxyl terminus of the HEV ORF2 protein has been used in a sensitive and specific EIA format for the detection and quantification of both acute and convalescent phase HEV specific IgG.<sup>93</sup> A synthetic gene encoding multiple linear immunodominant antigenic epitopes from ORF2 and ORF3 regions has been synthesized, expressed as a protein and used in a solid phase

EIA.<sup>94</sup> However recombinant proteins are more sensitive than synthetic antigens for detecting IgG anti HEV.<sup>95</sup> An analysis of IgM-specific EIAs indicated very high concordance when similar antigens produced in *E. coli* and insect cells were compared (concordance, 96.3%; kappa, 0.87).<sup>95</sup> ORF2 based EIAs were found to be significantly more sensitive than ORF3 based EIAs and the sensitivity varied from 18-100% especially in non endemic countries. EIAs based on HEV specific artificial recombinant mosaic protein composed of antigenic epitopes from ORF-2&3 are commercially available.

Current EIAs that measure IgM anti-HEV use the sandwich method of detection. Sensitivity is compromised when the corresponding IgG titers are disproportionately higher than those of the IgM antibodies, since the IgG competes for binding sites on the antigen. High titers of Rheumatoid Factor interfere with the IgM assay. Anamnestic response has also been noted. Recently a capture EIA format was shown to be more sensitive in detecting IgM anti-HEV especially in the presence of high concentrations of IgG antibodies.<sup>96</sup> In the IgM class capture system, competing IgG antibodies in the sample are eliminated at the beginning of the assay, thus enhancing the reaction between the IgM anti-HEV and the HEV antigen. The class capture method was more sensitive than the sandwich EIA when used to test clinical samples from two hepatitis E epidemics in Pakistan.

A new Immunochromatographic test has been developed for the rapid detection of IgM anti-HEV within 2-3 min with a specificity of 97% and sensitivity of 96%. The newly developed test is a reverse-flow immunochromatographic test and uses immobilized

mouse anti-human IgM antibodies for capturing the IgM antibodies in the tested samples. The presence of the captured IgM antibody specific to HEV is detected by the colloidal gold-labelled anti-HEV monoclonal antibody 4B2 premixed with the recombinant protein of ORF2.

Simultaneous detection of IgA and IgM antibodies to HEV was found to be highly specific for the diagnosis of acute HEV infection.<sup>97</sup>

The detection of HEV like particles and HEV RNA in faeces, bile, blood and liver has been accomplished by the use of immune electron microscopy (IEM) and the polymerase chain reaction (PCR). Several reverse transcriptase PCR assays (RT PCR) have been developed which can detect HEV RNA in faeces of most patients with acute hepatitis E during the initial few weeks.<sup>98</sup> However RT PCR for HEV RNA is less suitable than serologic identification of IgM anti-HEV for routine diagnosis because HEV RNA is degraded during faecal shedding of the virus and the short viraemic phase usually occurs before the disease is clinically apparent. Hence, EIAs for the detection of antibodies to HEV are the most convenient, inexpensive and suitable assays for diagnosis and tracking the infection.

***Prevention strategies:***

Based on seroprevalence studies, it has been estimated, that a third of the world's population has been infected with HEV. The poorer rural and urban populations of developing countries of Asia, the Middle East and Africa are at risk of contracting HEV

infection and would be candidates for a hepatitis E vaccine. Other groups at special risk of contracting HEV infection may be recipients of blood transfusions and patients on maintenance haemodialysis in regions where HEV is highly endemic.<sup>39, 99</sup> Groups at special risk of severe hepatitis E include pregnant women and patients with CLD. In industrialized countries, the military, visitors to HEV endemic regions, swine handlers and those who eat raw or uncooked, contaminated food (pork and shellfish) are at higher risk of infection.

In the absence of a viable HEV vaccine, improved sanitation and public hygiene and consumption of safe drinking water are the main stay of control of the disease.

*Passive immunoprophylaxis:*

Immunoprophylaxis trials of normal immune globulin in countries where HEV is endemic have not demonstrated statistically significant protection. The failure to demonstrate protection probably resulted from the relatively low titres of anti-HEV found in such globulin preparations. However direct demonstration of the protective efficacy of anti-HEV has come from studies in which, convalescent plasma or serum obtained from naturally infected patients or experimentally infected non human primates was infused into naive non human primates which were then challenged with HEV.<sup>100</sup> The immune globulin protected the animals against hepatitis. Further evidence that antibodies protect against HEV come from studies of monoclonal antibodies that can neutralize HEV. Thus, if an immune globulin preparation with a sufficient high titre of anti-HEV can be prepared, it would be efficacious in preventing HEV when administered before exposure.

*Active immunoprophylaxis :*

Among the HEV proteins, those encoded by ORF1 are non structural and not accessible to antibodies. These proteins are also among the least immunogenic of the virally encoded proteins of HEV. The protein encoded by ORF3 is immunogenic but is relatively short lived and relatively genotype specific and the antibody to the ORF3 protein does not neutralize HEV. Thus the protein encoded by ORF2, the capsid protein of the virus is the best candidate for a hepatitis E vaccine. It has a highly conserved sequence and antibody to it is long lived, cross reactive among diverse strains, neutralizes HEV in vitro and protects non human primates against HEV following challenge with virulent virus.<sup>95,100,80</sup>

HEV ORF2 proteins for vaccine development have been expressed as recombinant proteins in a variety of systems but principally from *E. coli* as fusion proteins or insect cells from baculovirus vectors. The first reported candidate vaccine was a fusion protein expressed in *E. coli* from a truncated ORF2 gene. This 440 amino acid protein was designated trip E-C2.<sup>101</sup> Another truncated ORF2 protein expressed in *E. coli* has proven to be useful both for serodiagnosis and as a candidate vaccine.<sup>102</sup> This protein, truncated to amino acid 394 at its amino terminus and to amino acid 607 at its carboxy-terminus, is said to form homodimers that express a conformational epitope, but only in its dimerized form.

Several candidate vaccines expressed from baculovirus vectors in insect cells have been studied. These vaccine candidates represent the various cleavage products derived from

the 72 kDa full length ORF2 protein when expressed from baculovirus in insect cells.

They are:

1. A 62 kDa protein encompassing amino acids 112-660 at the carboxy terminus of the protein.<sup>103</sup>
2. A 55-56 kDa protein spanning aminoacids 112-607 of the ORF2 protein derived from a Pakistani strain. (genotype I of HEV)<sup>100</sup>

Significant progress has been made in the development of DNA vaccines to HEV with better immune responses demonstrated to vaccination with ORF2 cDNA co-delivered with cytokine genes. A novel combination of two vaccination approaches using VLPs as vehicles for delivering heterologous DNA vaccine has been tried.<sup>104</sup>

Although hepatitis E vaccines consisting of 62 kDa (Genelabs), or 56 kDa (NIH) ORF2 proteins were both immunogenic and protective, the superior stability of the 56 kDa protein vaccine resulted in its selection by GlaxoSmithKline for clinical evaluation. The vaccine preparation contained purified 56 kDa capsid antigen derived from the HEV strain Sar-55 and expressed from baculovirus as recombinant protein in vaccine acceptable SF9 insect cells.<sup>105</sup> A phase II / III trial of the vaccine was carried out by the US army in members of the Nepalese military. The vaccine appears to protect against all four recognized genotypes of human HEV. Thus only one vaccine will be needed for world wide protection. It appears that three doses of vaccine would be necessary for optimum protection and periodic booster immunizations may be necessary as the half

life of anti-HEV appears to be shorter than that of antibodies against other viruses. Thus a hepatitis E vaccine appears to be feasible in the near future.

### ***Acute on Chronic Liver Failure:***

Acute on chronic liver failure remains an extremely poorly defined entity, largely because of the considerable heterogeneity in the mode of presentation. A working definition has been proposed by Jalan<sup>17</sup> and associates: “Acute deterioration in liver function over a period of 2-4 weeks usually associated with a precipitating event, leading to severe deterioration in clinical status, with jaundice and hepatic encephalopathy (HE) and / or hepatorenal syndrome (HRS)”. The numerous factors that may precipitate acute decompensation of CLD are divided into two groups. First, due to the effects of a known hepatotoxic factor such as superimposed hepatotropic virus infection, drug reaction, ingestion of a hepatotoxin or excessive alcohol consumption. Second, liver injury due to precipitating factors such as variceal bleed or sepsis.

### ***Prognosticating ACLF:***

The parameter best represented in prognostic models in CLD is the serum bilirubin. End organs, namely, the kidney and brain are frequently involved and determine outcome. The traditional Child Pugh score (CPS) not an ideal system because it ignores multi organ functioning and is not versatile in detecting changes on a day to day basis.<sup>106</sup> The Mayo end-stage liver disease (MELD) score is a continuous system and was found to be reliable for predicting outcome in patients with decompensated cirrhosis over a 3 month period.<sup>107</sup> While the MELD score and the CPS are liver specific scores, scores which

have been validated among a general population in an intensive care setting have also been applied. These takes into account parameters representing respiration, coagulation, cardiovascular and central nervous systems. The 2 commonly used scores are Acute Physiology and Chronic Health evaluation (APACHE) II<sup>108</sup> or the Sepsis-related Organ Failure Assessment (SOFA)<sup>109</sup>. Cholongitas et al, applied all these scores to a cirrhotic population admitted to an ICU with an aim to evaluate 6 week mortality and concluded that the MELD score and SOFA were similar in their discriminating ability to predict survival and better than APACHE II or Child Pugh scores.<sup>110</sup>

*Manifestations of ACLF:*

Liver : hyperbilirubinaemia, hypoalbuminaemia, coagulopathy and thrombocytopenia are invariably present.

Circulatory changes : this holds the centre stage in the development of ACLF. Any alteration of hepatic perfusion, in a patient with CLD, be it generalized hemodynamic change like sepsis or GI bleed or a local alteration due to portal vein thrombosis leads to acute decompensation. Increased cardiac output, a dilated and hyporesponsive peripheral circulation, increased portosystemic shunting and a reduced renal blood flow are seen. These phenomena are thought to be secondary to a reduction in vascular responsiveness and desensitization to vasoconstrictors or to the effects of vasodilating factors, or both.

Nitric oxide (NO), a profound vasodilator is a potential mediator in the hyporesponsiveness observed in CLD. NO is formed from *L*-arginine by calcium



dependant NO synthase (NOS). Cirrhotics have elevated renin and angiotensin II levels due to splanchnic arterial vasodilatation, resulting in arterial underfilling of the renal vascular vasculature, leading to avid renal sodium and water retention inspite of an expanded total plasma volume. Induction of NOS is the likely explanation for the splanchnic arterial vasodilation. This has been borne out in studies on cirrhotic patients where inhibition of NOS corrected the altered systemic haemodynamics and improved renal function and sodium excretion.<sup>111</sup> Inducible NOS (iNOS) in sepsis is dependant on cytokines like tumor necrosis factor  $\alpha$  and interleukin I  $\alpha$  and its activity is compartmentalized to the putrescent areas. It is possible that this compartmentalization, plays a role in the differential dilation of the splanchnic circulation with relative vasoconstriction of the renal and cerebral circulation in ACLF, especially when there is sepsis. Endothelial NOS (eNOS) is expressed by hepatic sinusoidal cells under basal conditions and has been shown to modulate portal pressure.

Kidneys: HRS is characterized by severe renal hypo perfusion due to an increase in renal vascular resistance. It is one of the most dangerous complications of ACLF. Renal vasoconstriction is secondary to activation of the renin-aldosterone-angiotensin axis, sympathetic nervous system and increased endothelin-1 expressed in the hepatic sinusoids resulting in afferent arteriolar vasoconstriction. Compensatory mechanisms mediated by prostaglandins fail to maintain renal perfusion. This scenario is worsened in sepsis where there is worsening of vasodilatation. Excessive diuretics reduce renal perfusion further and non steroidal anti inflammatory drugs further prostaglandin production.

Brain: Hepatic encephalopathy (HE) is a potentially reversible neuropsychiatric syndrome that occurs in patients with significant liver dysfunction and is characterized by altered sleep cycle, varying degrees of confusion and disorientation, asterixis, hyperreflexia and slowing of the dominant rhythm on electroencephalography. Ammonia remains central to the pathogenesis of HE. Recent studies in patients suggest cerebral oedema resulting in raised intracranial tension (ICT) can occur in CLD and that the pathophysiologic basis of HE in ACLF may be similar to that in acute liver failure (ALF) which has an inflammatory basis. Patients with sepsis syndrome had greater severity of encephalopathy and more raised ICT. A higher level of proinflammatory cytokines, possibly mediated by NO, has been noted with worse HE.

Metabolic: Metabolic changes have a role to play in the worsening seen in ACLF. UGI bleed is one such scenario. In patients with cirrhosis, ingestion of erythrocytes produces a larger increase in ammonia than ingestion of whole blood or plasma. The haemoglobin molecule is totally devoid of the branched chain amino acid (BCAA) isoleucine and large amounts of two other BCAAs, valine and leucine which have low biological value. Moreover leucine activates breakdown of BCAA producing ammonia and leading to a net catabolic state.

*Management:*

Prompt identification and treatment of precipitants when possible may improve short term mortality. Liver transplantation remains the only viable option in patients with

continued worsening decompensation or multi-organ failure. The role of liver assist devices as a bridge to transplant is controversial at present.

## **METHODOLOGY**

This was a retrospective study of patients who had acute Hepatitis E (HEV) infection with underlying chronic liver disease (CLD) who attended the hepatology outpatient services of the Christian Medical College Hospital, Vellore from January 2001 to June 2006.

Of 1768 patients who were clinically suspected to have HEV infection and underwent testing, 282 patients were ELISA IgM HEV positive. Of these, 47 patients had definite evidence of underlying CLD and were chosen for this study. (figure 6)

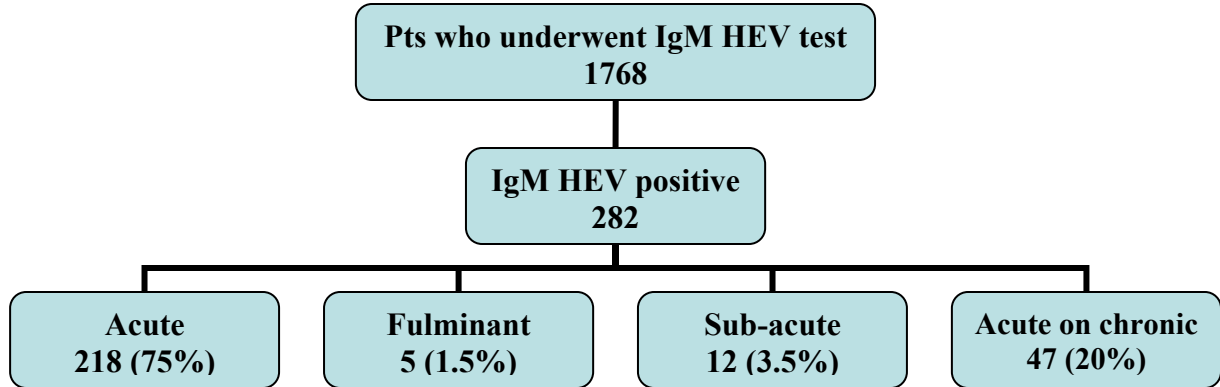


Figure 6: identification of patients who had HEV infection and CLD

Acute HEV infection was diagnosed using Immunoglobulin (Ig) M ELISA (Genelabs Diagnostics, Singapore), the assay detecting antibodies against recombinant proteins corresponding to open reading frames two and three of HEV.

The diagnosis of underlying CLD was made by:

- (a) patients having a prior diagnosis of cirrhosis based on clinical, biochemical, endoscopic, ultrasonographic and histologic findings or
- (b) presence of a nodular, irregular liver with either collaterals on ultrasonography or at least grade II varices on upper gastrointestinal endoscopy or histology.

Aetiological work up of CLD included HBsAg, anti-HCV, anti-nuclear antibodies (ANA), serum ferritin and a Wilson's work up. This comprised of serum ceruloplasmin, 24 hour urinary copper, liver biopsy, dry weight copper estimation by atomic absorption spectroscopy and slit lamp examination by an ophthalmologist to detect Kayser-Fleischer (KF) rings.

Other causes of acute hepatic decompensation such as superimposed acute hepatitis B virus (HBV) or acute hepatitis C virus (HCV) infection, hepatocellular carcinoma, acute hepatic or portal vein thrombosis and recent hepatotoxic drug ingestion were ruled out.

The modified Child Pugh score (CPS)<sup>106</sup> and Mayo end-stage liver disease (MELD)<sup>107</sup> score were calculated on all patients to prognosticate them.

The MELD score was calculated as follows:

$$3.8 \times \log(e) (\text{bilirubin mg/dl}) + 11.2 \times \log(e) (\text{INR}) + 9.6 \log(e) (\text{creatinine mg/dl})$$

The CPS was calculated as follows:

Measure	One point	Two points	Three points
<b>S. Bilirubin(mg/dl)</b>	<2	2-3	>3
<b>S. Albumin(mg/dl)</b>	>3.5	2.8-3.5	<2.8
<b>INR</b>	<1.7	1.71-2.20	>2.2
<b>Ascites</b>	None	↓ with diuretics	Refractory
<b>Encephalopathy</b>	None	Grade I-II	Grade III-IV

The clinical presentation, course in hospital and outcome of these patients were studied.

#### *Statistics:*

Data was reported as means with standard deviation or median with ranges (for continuous variables) and as frequencies and percentages (for categorical variables). Univariate analysis was performed using the independent sample *t* test for continuous variables which were normally distributed and the Mann Whitney test for continuous variables which were not normally distributed and Chi square test for categorical variables. To identify factors that were independently associated with mortality, multivariate logistic regression analysis was performed. The discrimination ability of identified variables to predict the outcome of patients (either dead or alive) was evaluated by using the area under a receiver operating characteristic (ROC) curve. This has the true positive and false

positive rates on the vertical and horizontal axes respectively. In this analysis, a model with an area under the curve (AUC) between 0.7-0.8 is considered clinically useful and between 0.8-0.9 as having excellent diagnostic accuracy. As the AUC approaches 1.0, the model approaches 100% sensitivity and specificity. Data was analyzed by statistical software SPSS (Statistical Package for Social Sciences, release 11.0, standard version; SPSS Inc). A p-value  $\leq 0.05$  was considered statistically significant. All p-values were 2-sided.

## **RESULTS**

Over five and a half years, there were 47 patients who had acute Hepatitis E virus infection (HEV) and underlying chronic liver disease (CLD). 35 (74%) were male and the mean age was  $38 \pm 18$  years (figure7).

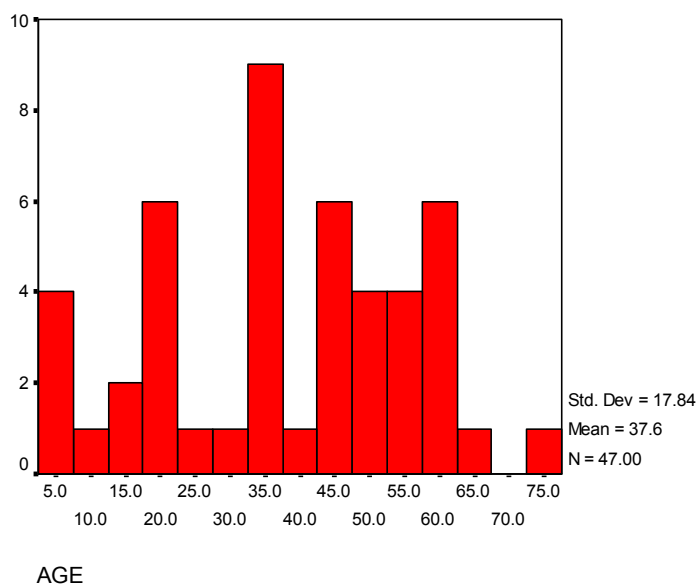
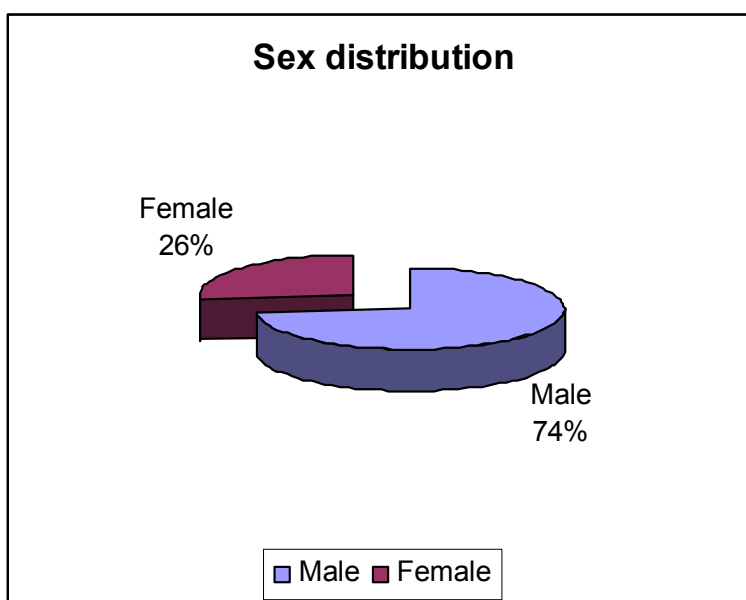


Figure 7: Demographic Profile





Only 9 (19%) patients had a prior diagnosis of CLD, the remaining patients presenting for the first time with decompensation. Wilson's disease was the most common cause of CLD and seen in 12 (25%) patients. Table II shows how the diagnosis of Wilson's disease was made in these patients. The other aetiologies (shown in figure 9) included Autoimmune 6 (13%), Hepatitis B virus infection 5 (11%), Ethanol 5 (11%) and Cryptogenic 10 (21%). Out of the remaining 9 (19%) patients, detailed aetiological evaluation remained incomplete at the time of death or discharge from hospital in 6, while 2 had cholestatic liver disease with secondary biliary cirrhosis and 1, extrahepatic portal vein obstruction with CLD.

**Table II - Diagnosis of Wilson's disease**

Age (years)	Ceruloplasmin (60-120IU/L)	KF ring	24hr U Cu (<100µg)	Liver biopsy	Dry wt Cu (15-55mg/Gm)
8	Nil	positive	590	cirrhosis	--
7	17	positive	4561	--	--
18	73	negative	275	Cu + bridging fibrosis	--
14	6	negative	540	--	--
34	112	positive	--	Cu + bridging fibrosis	--
36	70	negative	149	Cirrhosis	512
18	11	positive	900	Cirrhosis	--
41	20	--	87*	Cirrhosis	1000
7	63	--	2527	Cirrhosis	--
21	12	--	414	Cu + bridging fibrosis	--
21	19	positive	78*	cirrhosis	171
7	91	positive	907	--	--

**\* inappropriate sample collection**

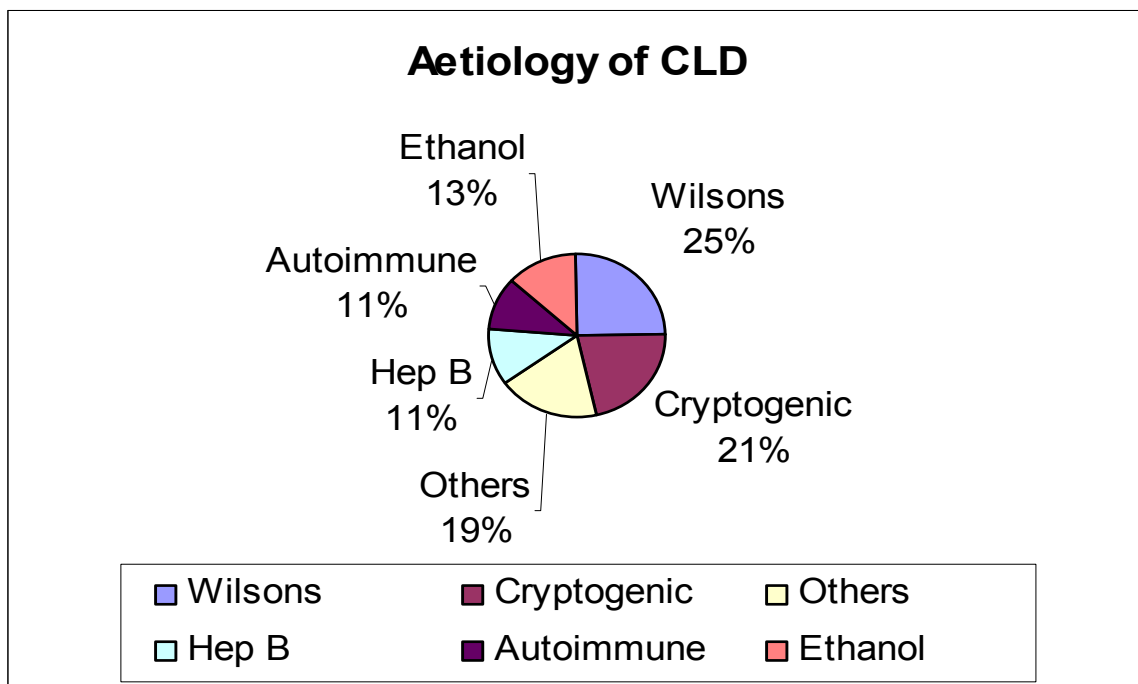


Figure 8: Aetiology of CLD

Forty-eight (98%) patients presented with jaundice with a median duration of 60(240) days. 39(83%) patients also had ascites with a median duration of 15(90) days. The median duration between onset of jaundice and development of ascites was 31(232) days. The median duration of the hospital stay was 11(59) days.

Biochemical investigations are listed in table III. Only 1 out of 38 (3%) patients tested positive for acute hepatitis A co-infection.

**Table III - Biochemical parameters**

<b>Variable</b>	<b>Mean + Standard deviation</b>
<b>Total bilirubin</b>	<b>16.9 <math>\pm</math>10.2 mg/dl</b>
<b>Serum albumin</b>	<b>2.3<math>\pm</math>0.5 mg/dl</b>
<b>AST</b>	<b>248<math>\pm</math>162 IU/ml</b>
<b>ALT</b>	<b>175<math>\pm</math>225 IU/ml</b>
<b>SAP</b>	<b>212<math>\pm</math>156 IU/ml</b>
<b>PT - INR</b>	<b>2.1<math>\pm</math>1.4</b>
<b>Creatinine (at admission)</b>	<b>1.1<math>\pm</math>0.8 mg/dl</b>
<b>Creatinine (highest)</b>	<b>1.7<math>\pm</math>1.5 mg/dl</b>
<b>IgM Anti HAV (N= 38)</b>	<b>1</b>

Upper gastrointestinal endoscopy was performed in 37 (79%) patients. As shown in figure 9, 16 (43%) patients had varices which were grade II or more (large), while 9 (24%) patients did not have any varices. On ultrasound examination, 29 (62%) patients had a normal liver size, while the remaining 18 (38%) patients either had a symmetrically shrunken liver or a significantly altered lobe ratio. A liver biopsy was performed in 20 (43%) patients with diagnostic intent; the transjugular method being the preferred route in 17 (85%) patients. One biopsy was a surgical specimen from an explanted liver during an orthotopic liver transplant while another was a post mortem specimen.

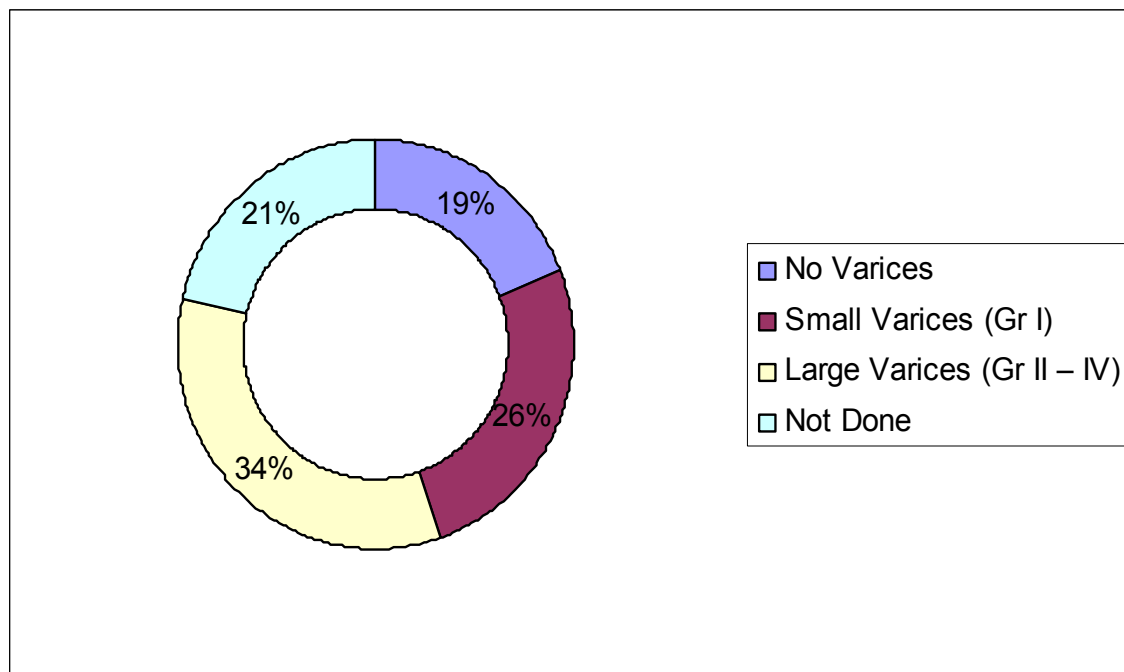


Figure 9: Oesophageal varices on endoscopy (performed in 74%)

The complications that the patients developed during their hospital stay are listed in table IV. 21 (45%) patients had hepatic encephalopathy. The grades of encephalopathy are shown in figure 10. 14 (30%) patients had spontaneous bacterial peritonitis, while 11 (23%) patients developed other infections like urinary, respiratory and skin (figures 11 and 12). Four patients had culture proven bacterial sepsis, the focus of which was not apparent.

**Table IV – Complications**

<b>Encephalopathy</b>	<b>21 (45%)</b>
<b>SBP</b>	<b>14 (30%)</b>
<b>GI Bleed</b>	<b>7 (15%)</b>
<b>Renal Failure</b>	<b>20 (43%)</b>
<b>Infection</b>	<b>25 (53%)</b>

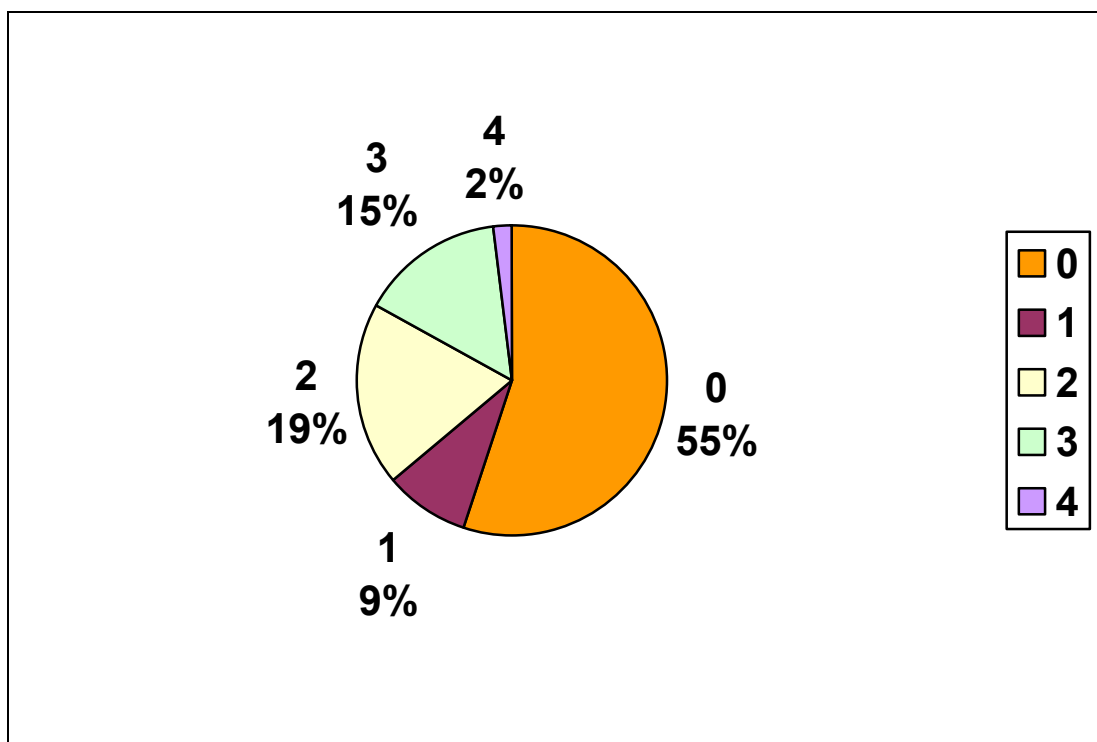


Figure 10: Grades of encephalopathy

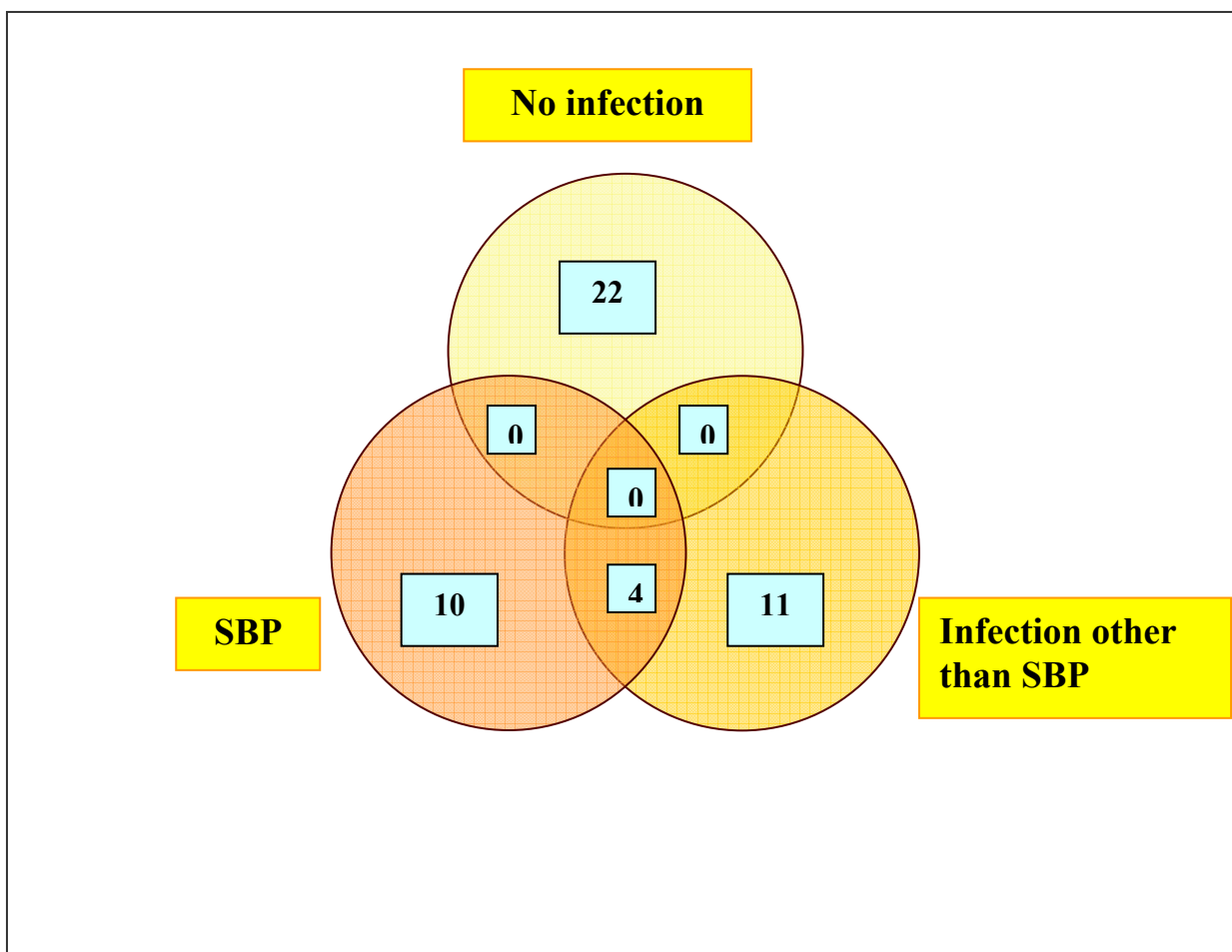


Figure 11: Infections

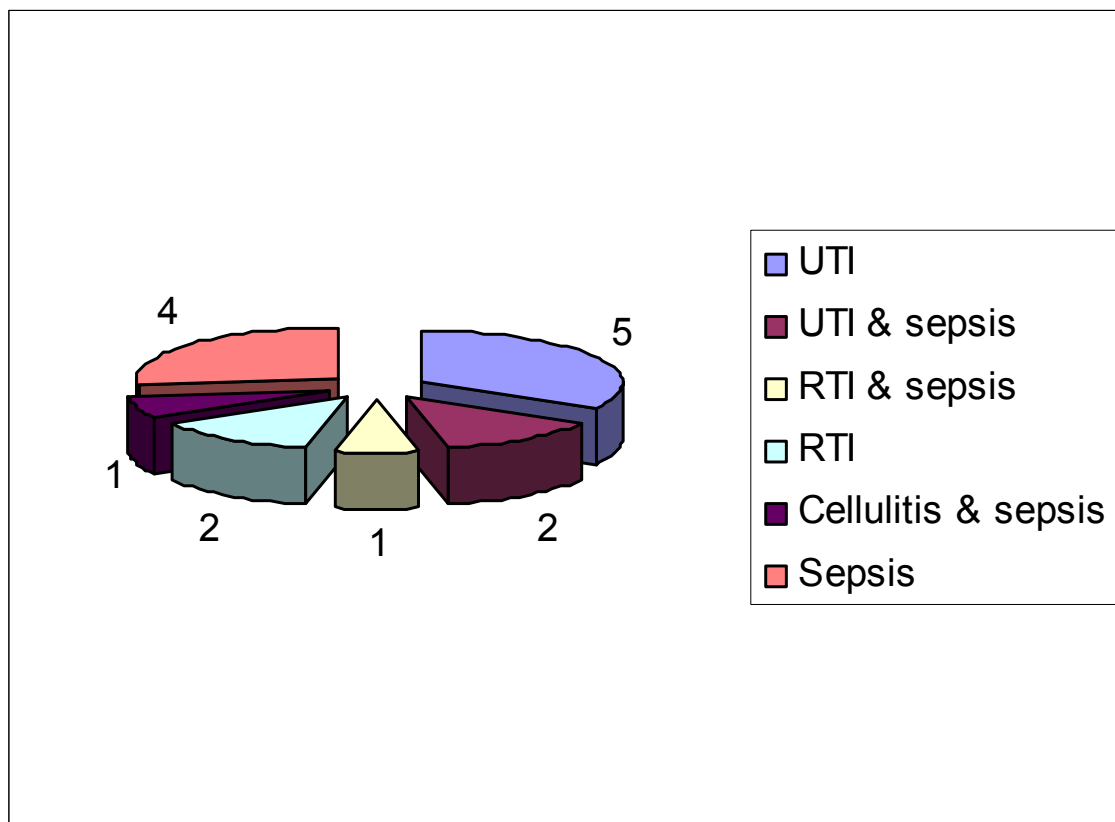


Figure 12: foci of infections

### Mortality

The overall mortality was high, 14 (30%) patients having died and 6 (13%) having been discharged in a terminal state (figure 13). These patients were also included in the mortality analysis. Of the 12 females and 35 males, 8 (67%) and 12 (34%) died respectively ( $p = 0.05$ ) (figure 14). Figure 15 shows the mortality according to the various aetiologies of CLD. The duration of jaundice at presentation or the duration between the onset of jaundice and development of ascites had no bearing on the outcome ( $p = 0.30$  and  $0.43$  respectively). On ultrasound examination, of the 29 (62%) patients with normal

liver size and 18 (38%) with shrunken liver, 9 (31%) and 11 (61%) died respectively ( $p = 0.04$ ).

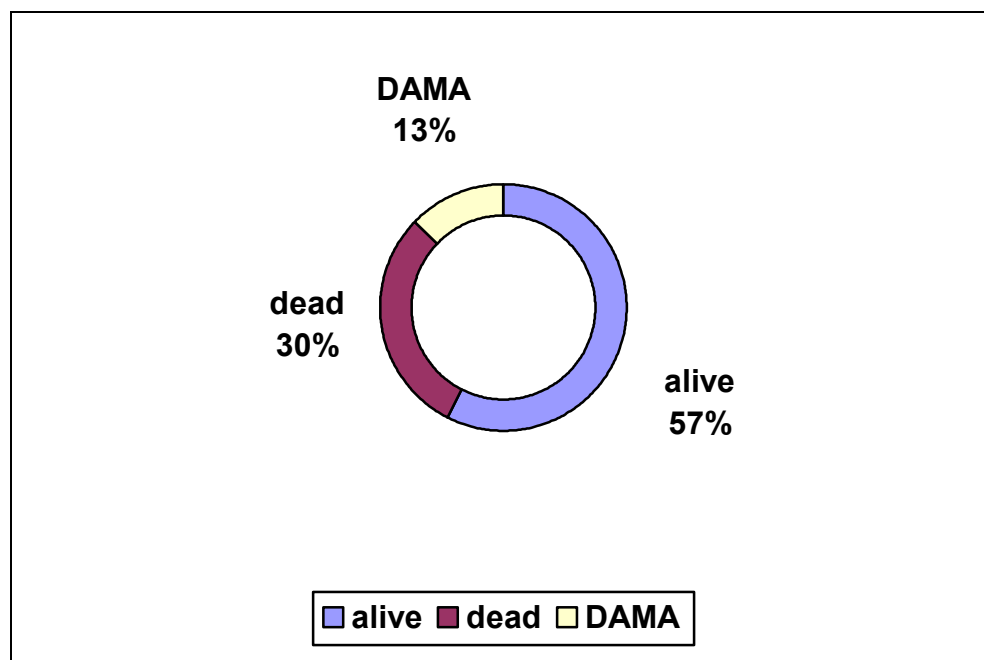


Figure 13: Outcome

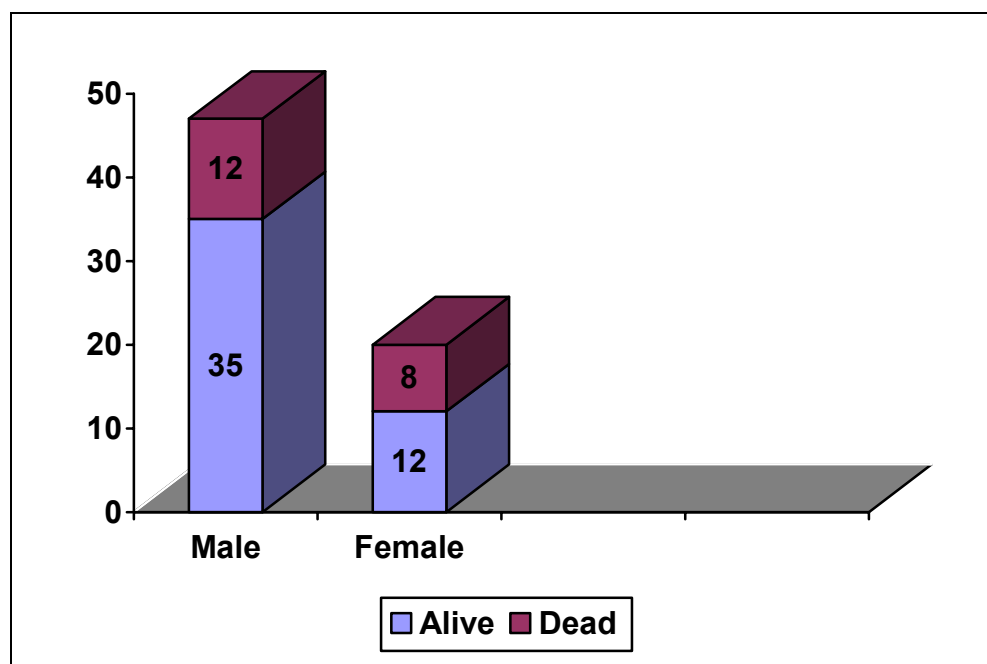


Figure 14: Mortality based on sex



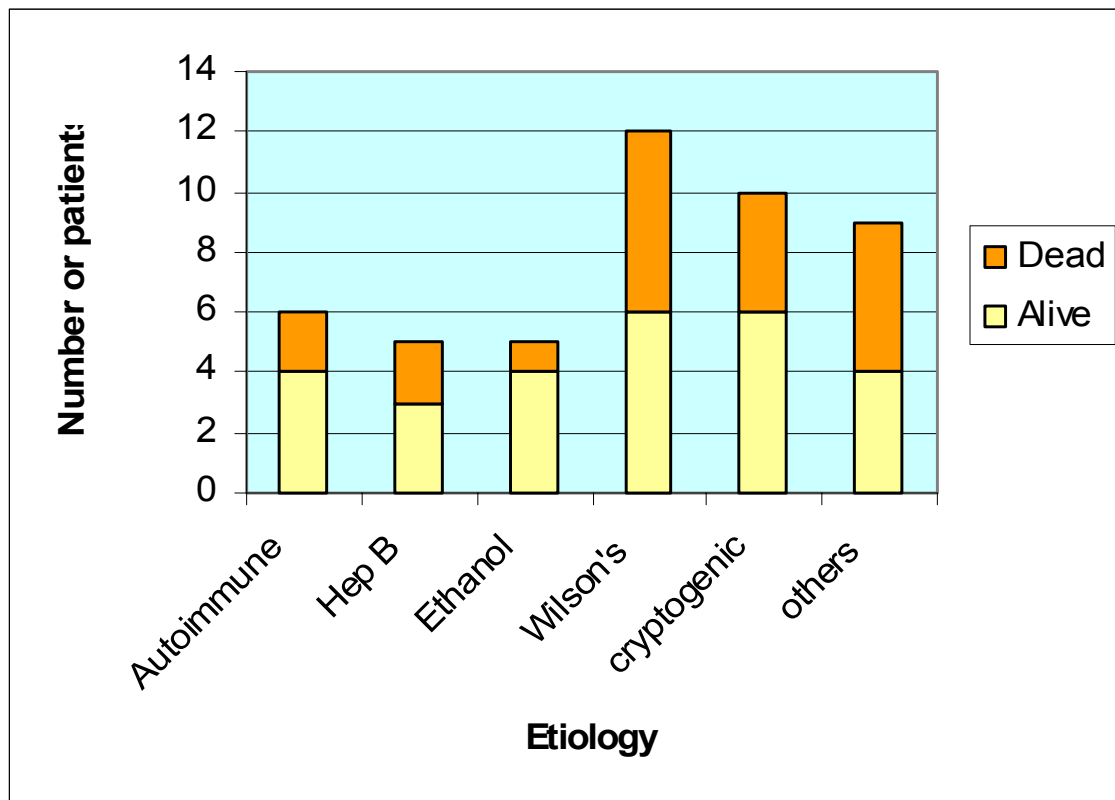


Figure 15: Mortality base on aetiology

*Factors associated with mortality: univariate analysis*

On univariate analysis, the presence of renal failure and hepatic encephalopathy were significantly associated with mortality ( $p < 0.001$  and  $p = 0.02$  respectively). Of the biochemical variables, serum bilirubin ( $p < 0.001$ ), serum creatinine ( $p < 0.001$ ), serum sodium ( $p < 0.001$ ) and the MELD score ( $p < 0.001$ ) had prognostic value. The factors affecting mortality are listed in table V(a) and table V(b).

**Table V(a) - Factors affecting mortality (univariate)**

<b>Variable</b>	<b>Alive (N=27)</b>	<b>Dead (N=20)</b>	<b>p value</b>
<b>Age (yrs)</b>	<b>34<math>\pm</math>17</b>	<b>42<math>\pm</math>18</b>	<b>0.13</b>
<b>Duration of jaundice (days)</b>	<b>60(240)*</b>	<b>30(239)</b>	<b>0.30</b>
<b>Jaundice to ascites interval (days , N=39)</b>	<b>30(224)*</b>	<b>55(231)</b>	<b>0.24</b>
<b>Shrunk liver on USG</b>	<b>7 (26%)</b>	<b>11 (55%)</b>	<b>0.04</b>
<b>Encephalopathy</b>	<b>8 (30%)</b>	<b>13 (65%)</b>	<b>0.02</b>
<b>SBP</b>	<b>8 (30%)</b>	<b>5 (25%)</b>	<b>0.73</b>
<b>Infections</b>	<b>12 (44%)</b>	<b>13 (65%)</b>	<b>0.16</b>
<b>Renal failure</b>	<b>4 (15%)</b>	<b>16 (80%)</b>	<b>&lt; 0.001</b>

\* median (range)

**Table V(b) - Factors affecting mortality (univariate)**

<b>Variable</b>	<b>Alive (N=27)</b>	<b>Dead (N=20)</b>	<b>p value</b>
<b>Bilirubin (mg/dl)</b>	<b>13.3<math>\pm</math>9</b>	<b>21.8<math>\pm</math>10</b>	<b>&lt; 0.001</b>
<b>S. albumin (g/dl)</b>	<b>2.2<math>\pm</math>0.6</b>	<b>2.3<math>\pm</math>0.4</b>	<b>0.80</b>
<b>ALT (IU/ml)</b>	<b>158<math>\pm</math>108</b>	<b>198<math>\pm</math>326</b>	<b>0.55</b>
<b>PT - INR</b>	<b>1.9<math>\pm</math>0.7</b>	<b>2.5<math>\pm</math>1.9</b>	<b>0.15</b>
<b>Creatinine (mg/dl)</b>	<b>0.9<math>\pm</math>0.4</b>	<b>2.6<math>\pm</math>1.8</b>	<b>&lt; 0.001</b>
<b>Sodium (mg/dl)</b>	<b>135<math>\pm</math>5</b>	<b>127<math>\pm</math>10</b>	<b>&lt; 0.001</b>
<b>MELD</b>	<b>20<math>\pm</math>7</b>	<b>32<math>\pm</math>10</b>	<b>&lt; 0.001</b>
<b>Modified CPS</b>	<b>11<math>\pm</math>2</b>	<b>12<math>\pm</math>2</b>	<b>0.07</b>

*Factors associated with mortality: multivariate analysis*

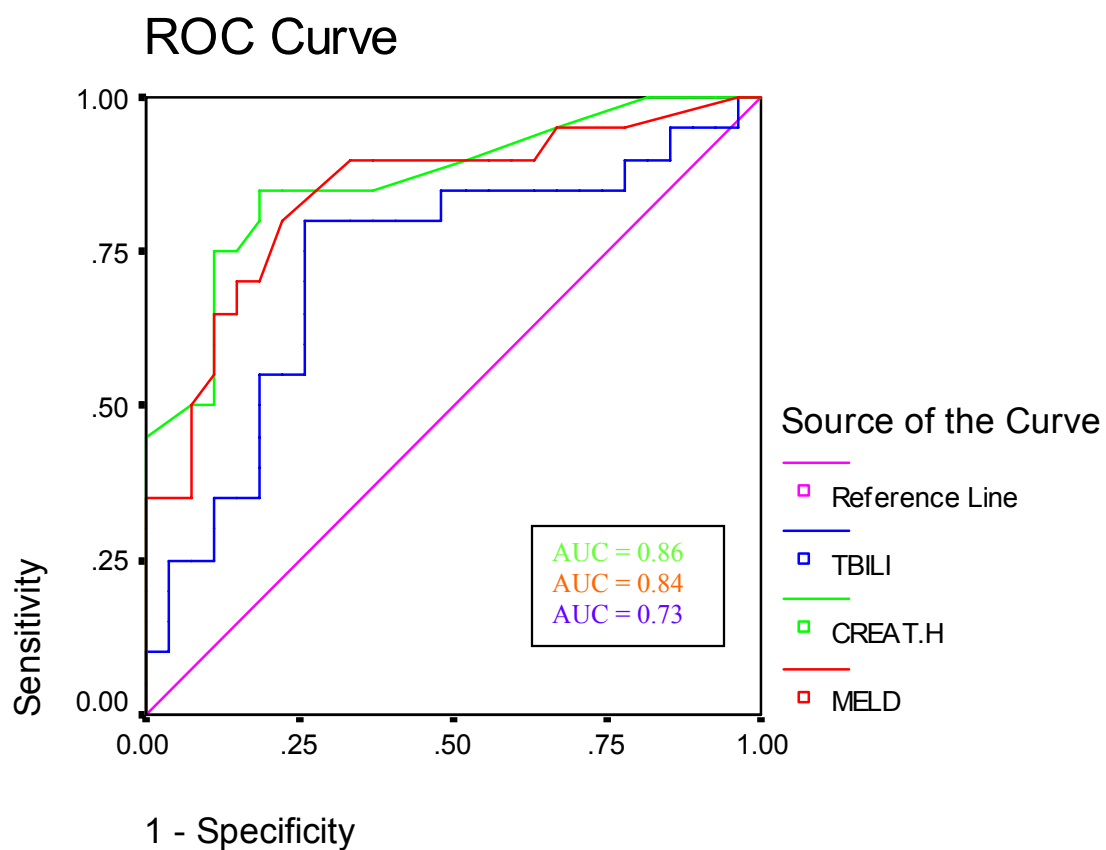
On multivariate logistic regression analysis, comparing the MELD score and serum creatinine separately with serum sodium and encephalopathy, the MELD score and serum creatinine were independently associated with mortality ( $p < 0.001$ ).

*Prognostic factors associated with mortality: receiver-operating characteristic (ROC) curves*

Based on the area under the ROC curves, serum creatinine had the best discriminative accuracy for mortality (AUC = 0.86), followed by the MELD score (AUC = 0.84) and serum bilirubin (AUC = 0.73) (Figure 16). Table VI shows the sensitivity and specificity at the cut off points of the above three variables as predictors of mortality.

**Table VI - Predictors of mortality**

<b>Variable</b>	<b>Value</b>	<b>Sensitivity (%)</b>	<b>Specificity (%)</b>
<b>Creatinine</b>	<b>1.3 mg/dl</b>	<b>80</b>	<b>82</b>
<b>MELD</b>	<b>23.5</b>	<b>80</b>	<b>78</b>
<b>Bilirubin</b>	<b>16.8 mg/dl</b>	<b>80</b>	<b>74</b>



Diagonal segments are produced by ties.

## **DISCUSSION**

This study confirms the already existing but sparse data that acute HEV superinfection in patients with chronic liver disease (CLD) produces severe decompensation and is associated with a high mortality rate.

The male to female ratio in this study was 4 : 1 in accordance with most other reports.<sup>6,8,43</sup> It is unclear whether this reflects a greater frequency of exposure among men due to the prevalent social milieu in India or a true difference in susceptibility.

The commonest aetiology of underlying CLD in this study was Wilson's disease. The prevalence of Wilson's among children in India varies from 7.5–10%.<sup>112,113</sup> In a study of liver diseases in childhood in which six medical colleges with organized paediatric gastroenterology and hepatology services participated, metabolic liver diseases constituted 8-43% of the reported CLD, of which Wilson's was the commonest metabolic cause of CLD.<sup>114</sup> The seroprevalence of HEV infection steadily increases from childhood, being 0-9% in the first decade and peaking at 40% between 16-25 years, after which it plateaus.<sup>115</sup> The seroprevalence of HEV infection in patients with CLD varies between 17-56% with the maximum prevalence being between the third and fifth decade.<sup>11,14,116</sup> Only 3 of the Wilson's patients in this study were over the age of 21 years.

This begs the question : are patients with Wilson's disease any more prone to the hepatic injury produced by HEV or is this simply a reflection of the higher chance of developing

HEV infection in an age group where Wilson's is the commonest metabolic cause of CLD ?

Acute liver failure is one of the myriad presentations of Wilson's and this presentation has been reported in a patient with acute HEV infection and co-existent Wilson's.<sup>117</sup> The majority of our patients presented for the first time with decompensation, very similar to the findings of Monga et al where seven out of ten patients were detected to have CLD during the current admission.<sup>13</sup> This underlines the importance of having a high index of suspicion for a superadded viral infection in a similar clinical setting. It is noteworthy that one patient in this study who presented for the first time with Wilson's disease and acute HEV infection underwent a live related orthotopic liver transplant successfully and is currently doing well.

Acute on chronic liver failure (ACLF) has been defined for us by Jalan and associates as an acute deterioration in liver function over a period of two to four weeks in a person with previous liver disease, usually associated with a precipitating event.<sup>17</sup> Indeed Kumar and colleagues, in their prospective case-control study of 14 CLD patients with acute HEV infection had included only cases with jaundice of less than four weeks duration.<sup>12</sup> However it was seen here that the median duration of jaundice at presentation was double that at eight weeks. Therefore the pre-occupation with time frames in defining the syndromes associated with liver failure, particularly ACLF, may warrant revision. More importantly, the duration of jaundice had no bearing on the outcome.

Only one of the patients had co-infection with the hepatitis A virus. This is hardly surprising considering that the seroprevalence of HAV infection in patients with CLD in an endemic region like India is 99%.<sup>11</sup>

As expected, a shrunken liver on ultrasound examination, an indicator of advanced cirrhosis was associated with mortality in this study. What was interesting however was the fact that more than 60% of the patients had normal sized livers and it was a liver biopsy, performed in 43% which helped clinch the diagnosis of underlying cirrhosis. This is especially important when the clinical scenario does not allow differentiation between ACLF and sub-acute hepatic failure (SAHF), an entity defined by Tandon et al, where the interval between the onset of jaundice and hepatic decompensation in the form of hepatic encephalopathy and / or progressive ascites is more than four weeks and less than twenty-four weeks.<sup>118</sup> This differentiation may have significant prognostic and therapeutic implications given the potentially reversible nature of ACLF if precipitants can be controlled.

The current study is the largest documented series of patients with CLD and serological evidence of acute HEV infection. The disproportionately high mortality that was observed is similar to what has been seen earlier from the Indian sub-continent, albeit in small case series. Hamid et al initially reported 4 cases of HEV superinfection with 1 death after which Jeyamani et al (6 deaths out of 9 cases), Kumar et al (2 out of 14) and Monga et al (3 out of 10) reported similarly in quick succession.<sup>14,11,12,13</sup> These studies



however failed to delineate the factors responsible for this dismal scenario, nor did they indicate how severe the decompensation was with respect to short term prognosis.

The modified Child Pugh score (CPS)<sup>106</sup> and Mayo end-stage liver disease (MELD) score<sup>107</sup> are liver specific prognostic scores. The MELD score has been validated for three month survival in cirrhotics and is currently used in donor liver allocation systems.<sup>119</sup> The Acute Physiology and Chronic Health Evaluation (APACHE II)<sup>108</sup> and Sequential Organ Failure Assessment (SOFA)<sup>109</sup> are ICU specific prognostic scores developed from general ICU populations, both providing clinical information on organ dysfunction. In a recent study comparing general and liver specific prognostic scores and evaluating six week mortality in 312 cirrhotics admitted to the ICU, MELD was similar to SOFA, both being better predictors of mortality than APACHE II or CPS.<sup>110</sup> In the same study, a MELD score of 22 had a sensitivity of 81% and specificity of 69% at predicting mortality.

In this study, the presence of renal failure and hepatic encephalopathy, both indicators of failing organ systems and hyponatremia were factors associated with mortality on univariate analysis. The MELD score and serum creatinine were independently associated with mortality. Alessandria et al<sup>120</sup> and Schepke et al<sup>121</sup> have reported similarly where MELD and hepato-renal syndrome I (HRS I) independently predicted survival in cirrhotics. A serum creatinine of > 1.5 gm/dl had an adverse impact on prognosis.<sup>120</sup> Schepke and associates have also demonstrated that HRS is associated with a worse prognosis than kidney dysfunction due to other causes including renal

parenchymal disease, volume depletion and infections. In this present study, serum creatinine had the best discriminative accuracy for mortality (AUC = 0.86) followed by MELD (AUC = 0.84) and serum bilirubin (AUC = 0.73). A serum creatinine of 1.3 and a MELD score of 24 had a sensitivity and specificity of around 80% for predicting mortality. Thus the development of renal failure in cirrhotic patients indicates a catastrophic reduction in survival probability, such that it is the predominant factor in end stage cirrhosis.

This study assessed for the first time MELD scores in patients with acute on chronic liver failure, the precipitating event being a superimposed hepatotropic viral infection. The MELD score is liver specific and contains surrogate variables related to more than one organ dysfunction. It proved to be an accurate prognostic indicator of patient mortality in the present study.

The Paediatric end-stage liver disease (PELD) score which additionally incorporates growth failure and age was proposed as a model for improving liver allocation in children awaiting transplantation<sup>122</sup> and has been validated as a predictor of post transplant survival.<sup>123</sup> This score was not applied to the 7 children in this study, because the aim was to obtain a comparative score between this small sub-group and the rest of the adult patients.

This study is not without its limitations. No comparisons were made with other forms of ACLF. Also, being retrospective in design, the study could not provide information on

the true incidence of HEV infection among patients with CLD. For this, a prospective case-controlled follow up study of patients with definite CLD in a HEV endemic region is required.

## **CONCLUSIONS AND RECOMMENDATIONS**

1. Acute HEV superinfection in patients with underlying CLD produces severe decompensation and is associated with a high mortality rate.
2. The MELD score and serum creatinine are the most important and accurate prognostic indicators of patient mortality in this setting.
3. A serum creatinine of 1.3 mg/dl and a MELD score of 24 were 80% sensitive and specific in predicting mortality.
4. The duration of jaundice did not affect the outcome.
5. Infections, including spontaneous bacterial peritonitis did not worsen the outcome.
6. A possible association between Wilson's disease and HEV superinfection requires further in depth study
7. A liver biopsy is crucial in conclusively establishing a diagnosis of underlying cirrhosis and must be performed when the clinical picture is unclear.
8. Obsessive attention to prevention and early aggressive management of treatable causes of renal failure may aid in improving the current short term mortality rates
9. In a developing country like India, with limited resources, one is duty bound to emphasize the importance of safe drinking water to all patients, especially those with CLD, in preventing HEV infection.

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## **PROFORMA (page i)**

Name :

Age :

Sex :

Hospital Number:

Jaundice: Present /Absent                      Duration of Jaundice:              (Days)

Ascites: Present / Absent                      Duration of Ascites:              (Days)

Duration of Jaundice to ascites:              (days)

Aetiology of Chronic liver disease (Please circle): Hepatitis B

Hepatitis C

Ethanol

Autoimmune

Wilson's

Cryptogenic

Others

Known case of Chronic liver disease: Yes/No

Course:        Alive / Dead / Discharged against medical advise (DAMA)

Encephalopathy:    Prevent/Absent

Grade of encephalopathy: 0 / 1 / 2 / 3 / 4

Spontaneous bacterial peritonitis: Present / Absent

GI Bleed: Present / absent

**PROFORMA (page ii)**

Varices: No / small varices (<Gr II) / Large varices (>Gr II) / not done

Infection (Other than SBP): Present / Absent

Type of Infection: Urinary: Present / Absent

(Culture proven) Respiratory: Present / Absent

Skin: Present / Absent

Sepsis: Present / Absent

Liver size on Ultrasound: Normal or Enlarged / Shrunken or asymmetric

Liver Biopsy Done: Yes / No

Route of Liver biopsy: Transjugular / Percutaneous / Surgical

Total Bilirubin (mg/dl) :

Serum Albumin: (mg/dl):

SGOT (IU/ml):

SGPT (IU/ml):

ALP (IU/ml):

PT (INR):

S. Creatinine (at admission):

S. Creatinine (highest):

S. Sodium (at admission):

S. Sodium (Highest):

IgM anti HBV: Done / Not done

Positive / Negative

Child Pugh Score:

Meld Score:

Follow up (days) (if available):